Method and Device for the Rapid Liquid Chromatographic Separation of Substance Mixtures and for the Identification of Substances

Specification

The invention relates to a process and a device for the rapid liquid-chromatographic separation of mixtures of substances and identification of substances according to the preambles of claims 1 and 5.

For example, the pharmaceutical research frequently encounters the problem of isolating pharmaceutically active substances from mixtures of substances. Thus, extracts of natural products or mixtures of substances produced by combinatorial chemistry are being tested for potential activity. Using mixtures of substances found to have activity, attempts are then made to isolate the active substances by means of complex separation procedures. Thereafter, the individual substances of the mixture isolated in this way are subjected to an activity test once more. The active individual substances being found are investigated for their structure so as to exclude active substances which might be already well-known. One drawback in this process is that when testing mixtures of substances, the activity of individual substances may be suppressed by superposition effects, leaving these substances undetected. Another drawback is possible simulation of activity by superposition effects, followed by a cost-intensive and useless search for these supposed active substances in the mixture of substances. Ultimately, it is disadvantageous that substances already well-known are excluded only after performing at

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least two tests for biological activity and after complex isolation procedures, which is very costly. As a rule, large quantities of material are required to perform these tests, i.e., separations have to be carried out on a preparative scale. With respect to capital investment, however, preparative units are more expensive compared to analytical units. Also, preparative units use considerably higher amounts of solvents and buffer substances during separation, rendering their operation costly and, in addition, giving rise to major problems with disposal and to environmental pollution.

The invention is based on the object of providing a device and a process for the liquid-chromatographic separation, isolation and identification of substances in an analytical and semi-preparative range, by means of which testing for activity of mixtures of substances is rendered unnecessary, and which enable separating mixtures of substances and isolating and identifying the individual substances more rapidly than is possible up to now.

Said object is accomplished through the characterizing parts of claims 1 and 5.

Advantageous developments are specified in the subclaims.

The invention has various advantages. Double testing of substances, namely, beforehand in the mixture of substances and after isolation, is no longer necessary. According to the invention, the complex and costly, and in part faulty first activity test of the mixtures of substances can be omitted. Instead, following combined isolation and identification, only potentially new active substances are subjected to further tests. One can do without costly treatment of substances already well-known, as is common practice up to now. The input of time and cost for detecting a

new active substance can be reduced substantially. In addition, this procedure is more reliable because the test results on unknown individual substances are unequivocal and also, all of the active substances present in the mixture will be detected.

The mixtures of substances to be investigated are processed in a two-stage separation wherein, because the separating columns and solid-phase extraction columns (collecting columns) are connected with the pump unit in a fashion according to the invention, parallel separation of multiple fractions from the first separation step is possible in the second chromatographic separation stage. Consequently, this apparatus works substantially more rapid and thus, more economical as compared to well-known two-stage apparatus.

The individual substances are identified using *per se* known direct computer-controlled comparison of chromatograms and spectra obtained from detectors, and of the retention range from the first separation step and the retention time from the second separation step with information on well-known substances in a data base. Ultraviolet absorption, mass spectrometry, light scattering, fluorescence, infrared spectroscopy, and nuclear magnetic resonance spectroscopy are possible as principles of detection and identification. It is also possible to use additional identification parameters such as source and origin of the sample. Because a smaller number of tests are required to identify the substances in the mixture and exclude substances already well-known, the plant can be dimensioned for analytical and semi-preparative scales. Analytical and semi-preparative units are much more economical with respect to initial cost and operation compared to preparative units commonly used so far. Owing to the reduced consumption of solvent and buffer substances, the process and device according to the

invention are environmentally beneficial as a result of re-
duced amounts of waste.

The invention will be illustrated in more detail with ref-
erence to the drawings and embodiments:

Fig. 1   is a schematic representation of the operating se-
        quence of equilibrating in the first separation
        step and washing of the feed column battery;

Fig. 2   is a schematic representation of the separation of
        a mixture of substances in the first separation
        step and the adsorption of fractions on the first
        collecting column battery;

Fig. 3   is a schematic representation of the separation of
        a mixture of substances in the first separation
        step and the adsorption of fractions on the second
        collecting column battery;

Fig. 4   is a schematic representation of the separation of
        a mixture of substances in the first separation
        step and the adsorption of fractions on the third
        collecting column battery;

Fig. 5   is a schematic representation of the equilibration
        of the separation column batteries of the second
        separation step;

Fig. 6   is a schematic representation of a parallel separa-
        tion of adsorbed fractions in the second separation
        step; and

Fig. 7   is a schematic representation of the equilibration
        of a collecting column battery.

Fig. 1 through Fig. 7 representatively illustrate the con-
figuration and flow scheme of a device according to the in-
vention, including a separation column and three downstream
separation lines.

A pump unit 2 comprised of three pumps 2.1 through 2.3 is
connected via 6-way 2-position valves 3.1 and 3.3 and 3-way
2-position valve 5.7 to a feed column battery 6, a separa-
tion column 10 for the first separation stage, and a second
separation stage comprised of three separation lines oper-
able in parallel, each of which having an upstream 6-way 2-
position valve 3.5, 3.6 and 3.7, respectively. In this way,
it is possible to convey the mobile phase in any desired
composition to any region of the device in a consecutive as
well as a parallel fashion.

Each separation line has a collecting column battery 7, 8
and 9 and a separation column battery 11, 12 and 13. Repre-
sentatively, the collecting column battery 7 includes the
collecting columns 7.1 through 7.6, and the separation col-
umn battery 11 includes the separation columns 11.1 and
11.2. The other two illustrated separation lines are iden-
tical in configuration. Other variants including more feed
columns 6.1 through 6.6 in the feed column battery 6, mul-
tiple separation columns 10, more than three collecting
column batteries 7, 8 and 9, each having more than six col-
lecting columns, and more than three separation column bat-
teries 11, 12 and 13 having more than six separation col-
umns per battery are also possible.

The operating sequence of the process according to the in-
vention will be described in an exemplary fashion below.
Samples of mixtures of substances are dissolved in a sol-
vent and added with an adsorbent each time. Subsequently,
the solvent is removed using a rotary evaporator so that
the adsorbent covered with sample material achieves flow-

ability properties. The adsorbents covered with the mixture
of substances are filled into the feed columns 6.1 through
6.6 of feed column battery 6 and secured in feed column
battery 6. The subsequent program sequence steps are con-
trolled by a software.

The separation column 10 is equilibrated according to Fig.
1. In a parallel operation, the air is removed from the
feed column battery 6. Via pump 2.3, 3-way 2-position valve
5.7 and 6-way 2-position valves 3.1 and 3.3, the air is re-
moved with water from one of the dry-filled feed columns
6.1 through 6.6 to be injected next. At the same time, the
separation column 10 is equilibrated with a suitable mo-
bile solvent via pump 2.1 and 6-way 2-position valves 3.1
and 3.3.

Fig. 2 illustrates the separation of the mixture of sub-
stances in the first separation stage on separation column
10 and the subsequent adsorption of the fractions in a
separation line including the collecting columns 7.1
through 7.6 of collecting column battery 7.

Once the air has been removed from one of the feed columns
6.1 through 6.6, the separation program is started. Ini-
tially, the 6-way 2-position valves 3.3 and 3.5 are
switched in position. Via a low-pressure valve unit 1 in-
cluding the low-pressure valves 1.1 through 1.3, the compo-
nents of the mobile phase can be fed into the system by
means of pump unit 2. The mobile phase is conveyed via the
low-pressure valve 1.1 of pump 2.1 and via pump 2.1, and
this system can be run both in an isocratic fashion and
with a gradient. Via 6-way 2-position valve 3.3 and 7-way
6-position valves 4.1/4.2, the mobile phase is conveyed by
pump 2.1 to that particular feed column 6.1 through 6.6
from which sample material is to be processed. The sample
to be separated is transferred from one of the feed columns

6.1 through 6.6 to the separation column 10. Via a 6-way 2-position valve 3.4 and detector 14.1, the separated sample components discharging from separation column 10 arrive at T-piece 17, where water is mixed into the mobile phase via pump 2.2 and 6-way 2-position valve 3.1. The amount of admixed water depends on the polarity of the substances to be separated. Now, as the polarity of the mobile is increased by the water, adsorption on collecting columns 7.1 through 7.6 of collecting column battery 7 is possible. Initially, adsorption is effected on the collecting column battery 7 via 6-way 2-position valve 3.5, the collecting columns 7.1 through 7.6 being loaded with fractions one by one.

Fig. 3 illustrates the adsorption of additional fractions on the collecting columns 8.1 through 8.6 of collecting column battery 8. Once all of the collecting columns of collecting column battery 7 are loaded with fractions, the 6-way 2-position valves 3.5 and 3.6 switch the collecting column battery 8 into the eluent stream. Now, the collecting columns 8.1 through 8.6 are loaded with fractions one by one.

Fig. 4 illustrates the adsorption of fractions on the collecting columns 9.1 through 9.6 of collecting column battery 9. Once all of the collecting columns 8.1 through 8.6 of collecting column battery 8 are loaded with fractions, the 6-way 2-position valves 3.6 and 3.7 switch the collecting column battery 9 into the eluent stream. Now, the collecting columns 9.1 through 9.6 are loaded with fractions one by one. In the next operating sequence step, the fractions adsorbed on the three collecting column batteries 7, 8 and 9 are eluted in parallel and separated further on the appropriately assigned separation column batteries 11, 12 and 13.

The separation column batteries 11, 12 and 13 are equili-
brated prior to each separation. Fig. 5 illustrates the
equilibration of separation column batteries 11, 12 and 13.
For equilibration, the mobile phase is conveyed to separa-
tion columns 11.1 and 11.2 of separation column battery 11
via pump 2.1 and 6-way 2-position valves 3.1 and 3.5, re-
spectively. From there, the mobile phase is conveyed to the
waste via 6-way 2-position valve 3.4, detector 14.1, and a
fraction collector 15.1. In a parallel operation, the sepa-
ration columns 12.1 and 12.2 of the separation column bat-
tery are equilibrated via pump 2.2, the 6-way 2-position
valves 3.1 and 3.6, a detector 14.2, and fraction collector
15.2. Likewise, the separation columns 13.1 and 13.2 are
equilibrated via pump 2.3, the 6-way 2-position valve 3.7
and 3-way 2-position valve 5.7, a detector 14.3, and a
fraction collector 15.3 in a parallel operation.

Fig. 6 illustrates the parallel separation of fractions ad-
sorbed on collecting column batteries 7, 8 and 9, using the
separation column batteries 11, 12 and 13. To initiate the
separation step, the mobile phase is conveyed to the col-
lecting column battery 7 via pump 2.1 of pump unit 2 and
6-way 2-position valves 3.1 and 3.5. The first eluted frac-
tion from collecting column battery 7 (e.g. from collecting
column 7.1) is passed to separation column battery 11 via
6-way 2-position valve 3.5. There, one of the separation
columns 11.1 or 11.2 can be switched in, optionally in a
software-controlled fashion. Subsequently, the separated
components are passed to the detector 14.1 via 6-way 2-po-
sition valves 3.5 and 3.4. The software in the electronic
control unit assesses the signals by peak detection, di-
recting the separated components into the appropriate vials
of fraction collector 15.1. Simultaneously, time control of
the fraction collector 15.1 is also possible. Such time
control can be activated automatically in case no peak
passes the detector.

In a parallel operation, the mobile phase is conveyed to the collecting column battery 8 via pump 2.2 and 6-way 2-position valves 3.1 and 3.6. The first eluted fraction from collecting column battery 8 (e.g. from collecting column 8.1) is passed to separation column battery 12 via 6-way 2-position valve 3.6. There, one of the separation columns 12.1 or 12.2 can be switched in, optionally in a software-controlled fashion. The separated components are passed to the detector 14.2. In this case as well, the software assesses the signals by peak detection, subsequently directing the separated components into the appropriate vials of fraction collector 15.2. Similarly, the fraction collector 15.2 can be operated in a time-controlled fashion. Such time control can be activated automatically in case no peak passes the detector.

In parallel to the operations in two separation lines, the third separation line is activated with respect to initiating the separation step. To this end, the mobile phase is passed to collecting column battery 9 via pump 2.3 and 3-way 2-position valve 5.7 and 6-way 2-position valve 3.7. The first eluted fraction from collecting column battery 9 (e.g. from collecting column 9.1) is passed to separation column battery 13 via valve 3.7. There, one of the separation columns 13.1 or 13.2 can be switched in, optionally in a software-controlled fashion. The separated components are passed to the detector 14.3. The downstream fraction collector 15.3 is controlled as described above. After each one of the first fractions has been processed in parallel, the separation column batteries 11, 12 and 13 are re-equilibrated (cf., Fig. 5) for preparation and in order to separate the next fractions. Subsequently, the 7-way 6-position valves 4.3/4.4, 4.5/4.6 and 4.7/4.8 on the collecting column batteries 7, 8 and 9 are switched over, so as to enable processing of the second fractions as illustrated in

Fig. 6. These operations are continued until all of the fractions have been processed.

Fig. 7 illustrates the equilibration of the collecting columns 7.1 through 7.6 of collecting column battery 7. In this program sequence step, the collecting columns 7.1 through 7.6 are washed with water, thereby being prepared for the next cycle. This is done in a sequential fashion via pump 2.2, 6-way 2-position valves 3.1, 3.5, 3.6, 3.7, and 7-way 6-position valves 4.3/4.4 of collecting column battery 7. Equilibration of the collecting column batteries 8 and 9 is performed in an analogous manner. The 6-way 2-position valves 3.5 and 3.6 are switched in position, and the collecting columns 8.1 through 8.6 are equilibrated via pump 2.2, 6-way 2-position valves 3.1, 3.5, 3.6, 3.7, and 7-way 6-position valves 4.5/4.6 of collecting column battery 8. Subsequently, the 6-way 2-position valves 3.6 and 3.7 are switched in position, and the collecting columns 9.1 through 9.6 are equilibrated via pump 2.2, 6-way 2-position valves 3.1, 3.5, 3.6, 3.7, and 7-way 6-position valves 4.7/4.8 of collecting column battery 9. Following this program sequence, the 7-way 6-position valves 4.1/4.2 of feed battery 6 are switched over to the next feed columns (e.g. 6.2), and the entire program cycle is restarted (Sequence step 1: Equilibration of separation column 10 and venting of feed column 6.2, illustrated in Fig. 1, etc.).

After processing the second sample, the next feed column 6.3 can be switched in the eluent stream. Because sample feed columns having undergone processing can be replaced by new ones anytime, continuous operation with an unlimited number of samples is possible.

During the first and second separation steps, chromatograms, retention data and spectra are collected via detectors 14.1, 14.2 and 14.3, processed directly in a com-

puter, and compared with data of known substances. Thus, known substances can be identified and sorted out already in on-line mode. In doubtful cases, additional data obtained off-line following separation and isolation can be used for identification.

Reference list

1   Low-pressure valve unit
1.1   Low-pressure valve
1.2   Low-pressure valve
1.3   Low-pressure valve
2   Pump unit
2.1   Pump
2.2   Pump
2.3   Pump
3   6-way 2-position valve
3.1   6-way 2-position valve
3.3   6-way 2-position valve
3.4   6-way 2-position valve
3.5   6-way 2-position valve
3.6   6-way 2-position valve
3.7   6-way 2-position valve
4   7-way 6-position valve
4.1   7-way 6-position valve
4.2   7-way 6-position valve
4.3   7-way 6-position valve
4.4   7-way 6-position valve
4.5   7-way 6-position valve
4.6   7-way 6-position valve
4.7   7-way 6-position valve
4.8   7-way 6-position valve
5   3-way 2-position valve
5.1   3-way 2-position valve
5.2   3-way 2-position valve
5.3   3-way 2-position valve
5.4   3-way 2-position valve
5.5   3-way 2-position valve
5.6   3-way 2-position valve
5.7   3-way 2-position valve

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6	Feed column battery
5.1	Feed column
5.2	Feed column
5.3	Feed column
5.4	Feed column
5.5	Feed column
5.6	Feed column
7	Collecting column battery
7.1	Collecting column
7.2	Collecting column
7.3	Collecting column
7.4	Collecting column
7.5	Collecting column
7.6	Collecting column
8	Collecting column battery
8.1	Collecting column
8.2	Collecting column
8.3	Collecting column
8.4	Collecting column
8.5	Collecting column
8.6	Collecting column
9	Collecting column battery
9.1	Collecting column
9.1	Collecting column
9.2	Collecting column
9.3	Collecting column
9.4	Collecting column
9.5	Collecting column
9.6	Collecting column
10	Separation column
11	Separation column battery
11.1	Separation column
11.2	Separation column
12	Separation column battery
12.1	Separation column
12.2	Separation column

13	Separation column battery
13.1	Separation column
13.2	Separation column
14	Detectors
14.1	Detector
14.2	Detector
14.3	Detector
15	Fraction collector
15.1	Fraction collector
15.2	Fraction collector
15.3	Fraction collector
16	Waste
16.1	Waste
16.2	Waste
17	T-piece